

## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 06 NOV 2001

WIPO PCT

Applicant's or agent's file reference RUT.00-0038	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US00/25930	International filing date (day/month/year) 21 SEPTEMBER 2000	Priority date (day/month/year) 21 SEPTEMBER 1999
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

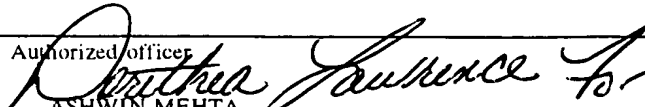
2. This REPORT consists of a total of 5 sheets.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 23 APRIL 2001	Date of completion of this report 09 OCTOBER 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer  ASHWIN MEHTA
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US00/25930

## I. Basis of the report

## 1. With regard to the elements of the international application:\*

- ☒ the international application as originally filed
- ☒ the description:  
pages 1-54, as originally filed  
pages NONE, filed with the demand  
pages NONE, filed with the letter of \_\_\_\_\_
- ☒ the claims:  
pages 55-59, as originally filed  
pages NONE, as amended (together with any statement) under Article 19  
pages NONE, filed with the demand  
pages NONE, filed with the letter of \_\_\_\_\_
- ☒ the drawings:  
pages 1-20, as originally filed  
pages NONE, filed with the demand  
pages NONE, filed with the letter of \_\_\_\_\_
- ☒ the sequence listing part of the description:  
pages NONE, as originally filed  
pages NONE, filed with the demand  
pages NONE, filed with the letter of \_\_\_\_\_

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.  
These elements were available or furnished to this Authority in the following language \_\_\_\_\_ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in printed form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

- ☒ the description, pages NONE
- ☒ the claims, Nos. NONE
- ☒ the drawings, sheets/fig. NONE

5. ☐ This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).\*\*

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

\*\*Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US00/25930

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

## 1. statement

Novelty (N)

Claims 1-22 YESClaims NONE NO

Inventive Step (IS)

Claims 1-22 YESClaims NONE NO

Industrial Applicability (IA)

Claims 1-22 YESClaims NONE NO

## 2. citations and explanations (Rule 70.7)

Claims 1-22 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest the use of a site-specific recombination system in plastids. The claims have the industrial applicability of removing undesirable marker genes from economically important transgenic crop plants.

----- NEW CITATIONS -----

NONE

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 1-22 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): the recitation "optional plastid targeting transit sequence" in lines 6-7 of claims 1 and 13 render the claims indefinite. It is not clear why the presence of the plastid targeting transit sequence is optional, given that the claimed method is for site-specific recombination that occurs in the plastid. How can the recombinase direct this reaction if it is not present in the plastid? There would be no utility for the invention if the plastid targeting sequence is not present.

Claims 1-22 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): the recitation "flanking plastid targeting nucleic acid sequences which facilitate homologous recombination into said plastid genome" in lines 5-7 of step b) of claim 1, and lines 4-6 of step b) of claim 13 renders the claims and those claims dependent thereon indefinite. Plastid targeting sequences do not facilitate homologous recombination. This recitation appears to be in error.

Claims 1-12 and 21 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): the recitation "predetermined target sequence" in the last two lines of claim 1 renders the claims indefinite. There is no antecedent basis for the recitation.

Claims 12 and 20 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): The claims are indefinite because it is not clear what the other components are that make up the site-specific recombination system. The metes and bounds of the claims are not clear.

Claims 13-20 and 22 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): the recitation "plant cells from step e)" in Step f) of claim 13 renders the claim and those dependent thereon indefinite. Step e) of claim 13 results in a regenerated plant. The cells are from step d). It is therefore not clear what cells step f) is referring to.

Claim 15 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claim is indefinite for the following reason(s): the claim is a "Markush" type claim that used improper Markush terminology. An --and-- should appear before "ribosomal" in line 4 of the claim.

(Continued on Supplemental Sheet.)

**Supplemental Box**

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

**CLASSIFICATION:**

The International Patent Classification (IPC) and/or the National classification are as listed below:

IPC(7): C12N 5/04, 15/09, 15/63, 15/64, 15/65, 15/67, 15/82, 15/87, 15/90; AO1H 1/00, 5/00 and US Cl.: 435/69.1, 69.7, 320.1, 410, 419, 468; 800/278, 287, 288, 295, 298, 303

**VIII. CERTAIN OBSERVATIONS ON THE APPLICATION (Continued):**

Claim 19 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims is indefinite for the following reason(s): the claim is indefinite because it is not clear what regenerated plant the claim is referring to. A plant is regenerated in step e) of claim 13. However, this is not the last step of the method. It is therefore not clear that it is the plant of step e) of claim 13 that claim 19 is referring to.

Claims 21 and 22 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): the claims are indefinite because they do not indicate that the progeny plants have inherited the site-specific recombination system from the parent plant. The genetic make-up of the progeny is therefore not clear.

## PATENT COOPERATION TREATY

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner  
 US Department of Commerce  
 United States Patent and Trademark  
 Office, PCT  
 2011 South Clark Place Room  
 CP2/5C24  
 Arlington, VA 22202  
 ETATS-UNIS D'AMERIQUE  
 in its capacity as elected Office

<b>Date of mailing (day/month/year)</b> 16 July 2001 (16.07.01)	
<b>International application No.</b> PCT/US00/25930	<b>Applicant's or agent's file reference</b> RUT.00-0038
<b>International filing date (day/month/year)</b> 21 September 2000 (21.09.00)	<b>Priority date (day/month/year)</b> 21 September 1999 (21.09.99)
<b>Applicant</b> MALIGA, Pal et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:  
 23 April 2001 (23.04.01)

☐ in a notice effecting later election filed with the International Bureau on:  
 \_\_\_\_\_

2. The election ☒ was  
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Claudio Borton Telephone No.: (41-22) 338.83.38
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## PCT

NOTICE INFORMING THE APPLICANT OF THE  
COMMUNICATION OF THE INTERNATIONAL  
APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

From the INTERNATIONAL BUREAU

To:

RIGAUT, Kathleen, D.  
Dann, Dorfman, Herrell and Skillman  
Suite 720  
1601 Market Street  
Philadelphia, PA 19120  
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year) 29 March 2001 (29.03.01)		
Applicant's or agent's file reference RUT.00-0038		IMPORTANT NOTICE
International application No. PCT/US00/25930	International filing date (day/month/year) 21 September 2000 (21.09.00)	
		Priority date (day/month/year) 21 September 1999 (21.09.99)
Applicant RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY et al		

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:

AU,KP,KR,US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

AE,AG,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CR,CU,CZ,DE,DK,DM,DZ,EA,EE,EP,ES,  
FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,  
MN,MW,MX,MZ,NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,  
The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on  
29 March 2001 (29.03.01) under No. WO 01/21768

**REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)**

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

**REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))**

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO 34, chemin des Calmbettes 1211 Geneva 20, Switzerland	Authorized officer  J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/25930

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435;/69.1, 69.7, 320.1, 410, 419, 468; 800/278, 287, 288, 295, 298, 303

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, Agricola, Biosis, Caplus

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LYZNIK et al. FLP-Mediated Recombination Of FRT Sites In The Maize Genome. Nucl. Acids Res. 1996, Vol. 24, No. 19, pages 3784-3789, especially pages 3784-3787.	1-22
Y	DALE et al. Gene Transfer With Subsequent Removal Of The Selection Gene From The Host Genome. Proc. Natl. Acad. Sci. USA. December 1991, Vol. 88, pages 10558-10562, especially pages 10559-10561.	1-6, 10-13, 16, 17, 19-22
Y	BAYLEY et al. Exchange Of Gene Activity In Transgenic Plants Catalyzed By The Cre-Lox Site-Specific Recombination System. Plant Molecular Biology. 1992, Vol. 18, pages 353-361, especially pages 356-358.	1-22

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

06 NOVEMBER 2000

Date of mailing of the international search report

04 DEC 2000

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

  
ASHWIN MEHTA

Telephone No. (703) 308-0196



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/25930

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LYZNIK et al. Heat-Inducible Expression Of FLP Gene In Maize Cells. Plant J. 1995, Vol. 8, No. 2, pages 177-186, especially pages 178-181.	1-22
Y	SHANKLIN et al. The Stroma Of Higher Plant Plastids Contain ClpP and ClpC, Functional Homologs Of Escherichia coli ClpP and ClpA: An Archetypal Two-Component ATP-Dependent Protease. Plant Cell. October 1995, Vol. 7, pages 1713-1722, especially pages 1714-1715 and 1720.	15
Y	SVAB et al. High-Frequency Plastid Transformation In Tobacco By Selection For A Chimeric aadA Gene. Proc. Natl. Acad. Sci. USA. February 1993, Vol. 90, pages 913-917, especially pages 914-915.	1, 13

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/25930

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (7):

C12N 5/04, 15/09, 15/63, 15/64, 15/65, 15/67, 15/82, 15/87, 15/90; A01H 1/00, 5/00

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435;/69.1, 69.7, 320.1, 410, 419, 468; 800/278, 287, 288, 295, 298, 303